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T cell subset alterations in idiopathic glomerulonephritis

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SUMMARY

Peripheral blood lymphocytes from 15 healthy controls and 59 patients with idiopathic glomerulonephritis were studied to determine whether an imbalance exists among human T cell subsets in these diseases. Twenty of the patients studied had a minimal change nephropathy (10 with nephrotic syndrome and 10 in sustained remission); 27 had a membranous glomerulonephritis (12 with nephrotic syndrome, six with isolated proteinuria and nine in complete remission); 12 patients had an IgA glomerulonephritis with heamaturia and mild proteinuria. Monoclonal antibodies directed at human T lymphocyte subsets termed OKT3, OKT4 and OKT8 were used in an indirect immunofluorescence assay in all cases. Patients with minimal change nephropathy, with or without nephrotic syndrome and patients with IgA glomerulonephritis showed mean values of $OKT3^+$ cells (total peripheral T cells), helper $OKT4^+$ cells, suppressor $OKT8^+$ cells and $OKT4^+/OKT8^+$ cell ratio, in the normal range. Only the group of patients with membranous glomerulonephritis and nephrotic syndrome presented a mean $OKT4^+/OKT8^+$ ratio greater than the normal group (percentages: $2{\cdot}43 \pm 0{\cdot}3$ vs $1{\cdot}6 \pm 0{\cdot}1$ s.e.m.; $P < 0{\cdot}02$). This increased ratio was due to a reduction in the $OKT8^+$ cell subset compared to the healthy subjects (percentages: $27{\cdot}6 \pm 2{\cdot}9$ vs $36{\cdot}8 \pm 1{\cdot}4$ s.e.m.; $P < 0{\cdot}01$). Our data shows that the functional lymphocyte disorders previously described in minimal change nephropathy and IgA glomerulonephritis are not due to a numerical imbalance of lymphocyte subsets. Such an imbalance of lymphocyte subsets was specifically observed in membranous glomerulonephritis with nephrotic syndrome. The true significance of this finding has to be clarified by longitudinal studies and functional tests.

INTRODUCTION

Several studies have been performed in the last decade to investigate the possible involvement of cell-mediated immunity in the pathogenesis of primitive glomerulonephritis. In 1974, Shalhoub's clinical observations suggested that an abnormality of the cellular immune system existed in minimal change nephropathy (MCN). The *in vitro* assays, subsequently performed to explore the cell-mediated immunity, have shown an inhibition of mitogen induced T lymphocyte blastogenesis (Moorthy, Zimmerman & Burkholder, 1976; Sasdelli *et al.*, 1981) and an enhanced suppressor cell activity in patients with MCN during the active phase (Wu & Moorthy, 1981). Ooi *et al.* (1980) have observed that a population of suppressor cells, responsible for a defective B cell function, exists in patients with membranous glomerulonephritis (MGN). Other authors have demonstrated, by func-

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tional tests, a weaker Con A-induced suppressor activity (Woo *et al.*, 1981) and a lower IgA-specific suppressor T cell activity in patients with IgA nephropathy (IgA GN) (Sakai, Nomoto & Arimori, 1979).

Previous studies have failed to demonstrate any abnormality in the number of peripheral T and B lymphocytes evaluated by Et, Ea and EAC rosette forming cells assay, which might explain the above functional findings in these nephropathies (Sasdelli *et al.*, 1981). More recently, however, several approaches have provided evidence for the existence of regulatory T cell subsets in man, and some methods are now available for their identification. These are based on the differential expression of either the receptor for the Fc fragment of immunoglobulin G and M, or specific cell surface antigens (Reinherz *et al.*, 1980). In particular, by using mouse hybridoma that secrete monoclonal antibodies, two main T cell subpopulations have been revealed in human peripheral blood, that seem to be implicated in the immune response modulation. One of these is found to be reactive with a monoclonal antibody termed OKT4 and seems to represent a helper subset of the T cell population; the other reacts with the OKT8 antibody and is considered a suppressor T cell subset. The OKT3 antibody seems to recognize most peripheral T cells (Dutton, 1975; Moretta, Mingari & Moretta, 1979).

Only a few reports however exist, up to now, on the distribution of lymphocyte subsets in human disease and, above all, in primitive and secondary glomerulonephritis (Morimoto *et al.*, 1980; Chatenoud & Bach, 1981). The aim of the present study therefore, was to analyse the distribution of $OKT3^+$, $OKT4^+$ and $OKT8^+$ cells in healthy subjects and in patients with IgA GN, MCN and MGN and to correlate possible modifications in the percentage and/or absolute number of T cell subsets with the degree of activity of these idiopathic glomerulonephritis.

MATERIALS AND METHODS

Patients. Fifty-nine blood samples collected in heparinized containers were obtained from the same number of patients with different idiopathic glomerulonephritis.

The diagnosis was established in all cases by history, clinical examination and renal biopsy. Renal specimens were studied by light microscopy and immunofluorescence in all cases and also by electron microscopy in 40 of them. The histological diagnosis was carried out according to Churg & Duffy (1973). When the clinical and laboratory data of patients with MCN and MGN was considered, 22 patients (10 with MCN and 12 with MGN) had a nephrotic syndrome (NS) at the time of the study; six patients with MGN had an isolated proteinuria and 19 (10 with MCN and nine with MGN) were in sustained remission. All 12 patients with IgA GN had a microscopic haematuria with mild proteinuria. In all the patients serum creatinine was less than 1·5 mg/dl. At the time of the study no patients had been treated with steroids and/or other immunosuppressive drugs or else the steroid schedule had been stopped for at least 4 weeks previously and cyclophosphamide for at least a year beforehand.

The control group consisted of 15 healthy volunteers with no evidence of renal or systemic disease.

The mean clinical and laboratory data of the subjects studied are reported in Table 1.

Methods. Thirty millilitres of heparinized venous blood were diluted and layered over a Ficoll-Hypaque density gradient. After centrifugation, the interface layer containing lymphocytes was washed twice in RPMI 1640 supplemented with 5% (v/v) heat-inactivated calf serum and 25 mM HEPES, and the cell count was adjusted to 5×10^6 cells/ml; 200 μl of the lymphocyte suspension were placed in each of three tubes. The tubes were then incubated for 30 min in an icewater bath with 5μl of OKT3, OKT4 and OKT8 antibodies (Ortho Pharm Corp., Raritan, New Jersey, USA) with the appropriate dilution. After incubation the cells were washed twice in cold phosphate-buffered saline (PBS), pH 7·4. Lymphocytes were then labelled with 50 μl of fluoresceinated goat anti-mouse (Bionetics Lab. Prod., Kensington, Maryland, USA), diluted 1:30. This reagent is said to be specific against mouse IgG (H + L chains). After 30 min at 4°C, the lymphocytes were washed twice in cold PBS and resuspended gently with a Pasteur pipette in PBS containing 30% v/v of glycerol. Membrane immunofluorescence was evaluated using a Leitz Orthoplan microscope equipped for

Table 1. Clinical and laboratory data of the subjects studied

	Patient No.	Age (Years)	Serum albumin (g/dl)	Serum cholesterol (mg/dl)	Proteinuria (g/24 hr)	Duration of NS before the study (months)
Normals	15	35·9 ± 8·4	4·3 ± 0·3	188·1 ± 34·2	0·09 ± 0·07	
Minimal change nephropathy						
With NS	10	23·5 ± 9·5	2·2 ± 0·4	307·8 ± 79·6	6·7 ± 3·0	1·1 ± 0·3
In remission	10	19·3 ± 13·5	4·0 ± 0·2	175·2 ± 26·2	0·1 ± 0·05	
Membranous glomerulonephritis						
With NS	12	47·2 ± 19·4	2·1 ± 0·4	365·5 ± 85·2	8·0 ± 3·8	10·5 ± 12·8
With proteinuria	6	34·6 ± 12·3	4·1 ± 0·5	226·5 ± 48·9	1·9 ± 1·1	
In remission	9	50·8 ± 14·8	4·2 ± 0·3	194·1 ± 39·1	0·1 ± 0·04	
IgA glomerulonephritis						
	12	35·1 ± 10·5	4·3 ± 0·2	191·9 ± 36·3	0·2 ± 0·3	

Values are mean ± s.d.

epifluorescence. Two hundred lymphocyte-like cells were counted, monocytes being excluded from the counts by morphological criteria. The staining of monocytes was performed with non-specific esterase (Yam, Li & Crosby, 1971) using α-naphtil butyrate as the substrate. As assay control we used fluorescein labelled goat anti-mouse in samples without monoclonal antibody.

The results were expressed as the percentage of each fluorescent T cell subset with respect to the total number of mononuclear cells present in each field. The ratio between OKT4 and OKT8 stained T cell percentage was also calculated to avoid possible errors of interpretation due to different cell separation conditions.

Statistical analysis. All results are expressed as arithmetic means ± mean standard error. The significance of the differences in mean values between each group and the group of healthy subjects was tested by Mann–Whitney *U*-test for non-parametric data.

RESULTS

Table 2 and Fig. 1 summarize the results of our study. No significant differences were found between the normal group and any of the other groups either for the total number of lymphocytes or for the $OKT3^+$ cell percentage and the absolute number.

Patients with MCN with or without NS showed mean values of $OKT4^+$ and $OKT8^+$ cells not significantly altered, compared to controls. The average of $OKT4^+/OKT8^+$ ratio was slightly lower in patients with MCN in remission than in healthy subjects and in patients with MCN and NS (percentages: 1·31 ± 0·1 versus 1·6 ± 0·1 and 1·59 ± 0·1 respectively), but the differences were not statistically significant. The lowering of $OKT4^+/OKT8^+$ ratio in remission was mainly due to an increase in $OKT8^+$ cells.

When patients with MGN were observed, a different behaviour existed between cases with and without NS. In fact in patients with NS, the mean $OKT4^+/OKT8^+$ ratio was greater than the average ratio observed in the control group (percentages: 2·43 ± 0·3 versus 1·6 ± 0·1; $P < 0·02$). The increased $OKT4^+/OKT8^+$ cell ratio was mainly due to a reduction in the $OKT8^+$ cells when compared with normal controls (percentage: 27·6 ± 2·9 versus 36·8 ± 1·4, $P < 0·01$; absolute numbers 627 ± 108 versus 826 ± 73, $P < 0·05$). When the disease activity decreased, the $OKT4^+/OKT8^+$ cell ratio diminished, due both to an increase in the $OKT8^+$ cell percentage and absolute number, and to a reduction in the $OKT4^+$ cells. In fact, in the group of patients with MGN in sustained remission the percentage of $OKT8^+$ cells reached 42·1 ± 4·2% and that of the $OKT4^+$ cells fell to 49·4 ± 5·2%. The examination of the individual data however demonstrated that two

Table 2. Peripheral lymphocyte counts and percentages and absolute numbers of T cell subsets in patients with idiopathic glomerulonephritis

		OKT3$^+$ cells		OKT4$^+$ cells		OKT8$^+$ cells		
	Lymphocytes per mmc	%	Absolute number per mmc	%	Absolute number per mmc	%	Absolute number per mmc	OKT8$^+$/ OKT4$^+$
Normals	2,208±131	70·7±2·2	1,574±125	58·7±1·9	1,311±107	36·8±1·4	826±73	1·6 ±0·1
Minimal change nephropathy								
With NS	2,256±264	71·5±2·1	1,569±347	61·6±2·3	1,390±185	38·8±2·6	875±97	1·59±0·1
In remission	2,345±123	69·9±3·4	1,601±402	57·1±3·7	1,336±102	43·4±2·2	1,017±57	1·31±0·1
Membranous glomerulonephritis								
With NS	2,205±254	73·5±2·1	1,688±232	58·6±3·9	1,292±195	27·6±2·9*	627±108‡	2·43±0·3†
With proteinuria	1,827±185	69·5±6·8	1,256±196	54·1±4·3	1,008±158	36·3±3·6	659±80	1·53±0·1
In remission	1,778±224	73 ±2·1	1,314±338	49·4±5·2‡	903±174‡	42·1±4·2	726±85	1·33±0·2
IgA glomerulonephritis	1,916±200	67·5±3·5	1,308±166	54·1±4·4	1,070±170	37·3±2·7	729±107	1·5 ±0·2

Values are mean±s.e.m. Statistical significance derived from the Mann–Whitney *U*-test for non-parametric data. Difference between each group and healthy subjects: * $P<0·01$; † $P<0·02$; ‡ $P<0·05$.

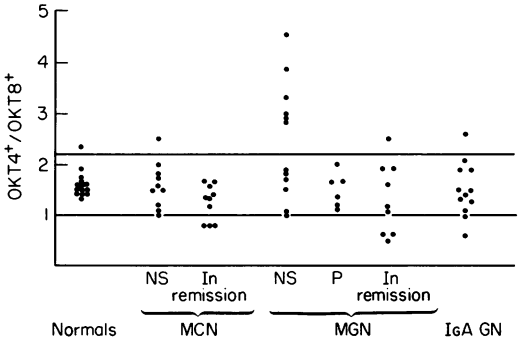

Fig. 1. Distribution of individual values of $OKT4^+/OKT8^+$ cell ratio in the healthy subject group and in patients with different glomerulonephritis at different phases of activity. Horizontal lines indicate the normal range (mean $\pm$ 2 s.d.). MCN = minimal change nephropathy; MGN = membranous glomerulonephritis; IgA GN = IgA glomerulonephritis; NS = nephrotic syndrome; P = isolated proteinuria.

different patterns of T cell subset behaviour existed even in the group of patients with MGN and NS. Six out of the 12 cases had a marked increase in the $OKT4^+/OKT8^+$ ratio, while the others showed an $OKT4^+/OKT8^+$ ratio in the normal range (Fig. 1). No significant difference existed between these two subgroups in mean values of age, duration of NS before the study, albuminaemia, cholesterolaemia and proteinuria.

For all the patients with IgA GN, none of the parameters studied were significantly different from those of the controls.

DISCUSSION

Various experimental studies and serological and immunohistological tests in man, would appear to demonstrate the key role of immunological mechanisms in the pathogenesis of most glomerulonephritis. For many years nephrologists and immunologists focused their researches on the abnormalities of the humoral immunity. The increasing evidence of a close relationship between the humoral and cell-mediated limbs of the immune system and the emerging data that show a possible direct involvement of cell-mediated immunity in the pathogenesis of some glomerulonephritis, have induced many authors to pay more and more attention to its dysfunctions as a primitive possible cause of these diseases.

Our and other authors' studies have shown that in MCN with NS there is a consistent defect of the lymphocyte response to mitogens (Moorthy *et al.*, 1976; Sasdelli *et al.*, 1981). There is complete agreement in literature on the observation that the lymphocyte hyporesponsiveness to lectins is not due to an alteration in the percentage and/or absolute number of T lymphocytes evaluated as Et and Ea rosettes (Sasdelli *et al.*, 1981). More recently, Chatenoud & Bach (1981) have studied, by means of specific monoclonal antibodies, the distribution of $OKT3^+$ cell (total peripheral T cells) and of the regulatory T cell subsets (helper $OKT4^+$ and cytotoxic/suppressor $OKT8^+$ cells) in MCN, to clarify whether a lymphocyte subset imbalance exists in this condition which might explain the above functional findings. The authors' report did not demonstrate any abnormality in the distribution of peripheral lymphocyte subsets in MCN. However, only two out of the six cases studied were in the active phase and four of the six were being treated with steroids when the test was performed. Our experience confirms in a larger series of patients with MCN, with or without NS, the absence of significant alterations in lymphocyte subset percentages and absolute numbers and the presence of a normal balance between $OKT4^+$ and $OKT8^+$ peripheral T cells. This observation agrees perfectly with our and other authors' hypothesis that the lymphocyte hyporesponsiveness in patients with MCN is due to a functional lymphocyte disorder (Sasdelli *et al.*, 1981). Whether this functional alteration is primitive, or rather due to inhibitory plasma factor(s) specific to MCN during the active phase, is not the concern of this study and has been discussed elsewhere (Sasdelli *et al.*, 1981).

As regards the findings in patients with MGN some observations can be drawn from our study. MGN may be due to glomerular entrapment of circulating immune complexes or to *in situ* immune complexes formation in the kidney (Evans, 1974; Van Damme *et al.*, 1978). The observations of Dixon, Feldman & Vazquez (1961) in chronic serum sickness nephritis in rabbits and those of Germuth & Rodriguez (1973) seem to suggest that membranous nephropathy occurs in subjects developing a very poor antibody response to antigens. In this way small sized immune complexes might be formed, which would be responsible for the typical histological and immunohistological pattern of the disease. According to this hypothesis, Ooi *et al.* (1980) recently described a reduced IgG and IgM production by polyclonally activated B lymphocytes from 11 patients with MGN. Our data and those of Chatenoud & Bach (1981), showing an increased $OKT4^+/OKT8^+$ cell ratio in MGN with a relative reduction of the suppressor cell subset versus the helper cell subset, seems to contrast with the above hypothesis. However, Ooi *et al.* (1980) also demonstrate, by functional tests, that the defective lymphocyte function was due to the presence of a monocyte suppressor cell population rather than to a T lymphocyte suppressor population, so that our results only apparently contrast with those of these authors. The true significance of these findings in MGN is controversial. In fact, Chatenoud & Bach (1981) observed an increased $OKT4^+/OKT8^+$ cell ratio in six out of 11 cases of MGN without NS and a very slight increase in this parameter in the only patient studied during NS. This favoured the hypothesis of a sustained immune stimulation in this disease. Our data in a greater number of patients showed that only the group of patients with MGN and NS had a mean $OKT4^+/OKT8^+$ cell ratio significantly greater than the normal control group. It was not possible to suggest that the increased $OKT4^+/OKT8^+$ cell ratio was related to NS *per se*, because the same ratio was normal in patients with biochemically comparable NS due to MCN. Moreover, there was no correlation between the duration of the NS before the study, the entity of nephrotic syndrome and the $OKT4^+/OKT8^+$ ratio. The patients with isolated proteinuria or in sustained remission had a normal lymphocyte subset balance. This normalization did not seem to be due to the previous therapy. In fact, no correlation was found between the $OKT4^+/OKT8^+$ ratio and treatment before the study. The values of the $OKT4^+/OKT8^+$ ratio in treated and untreated patients presented a considerable overlap and the percentage of treated and untreated patients was similar in the three subgroups of MGN with different activity of the disease. Therefore our results seem to suggest that a specific dysregulation of T cell subsets exists in patients with NS due to MGN. However our data showed a different behaviour of $OKT4^+/OKT8^+$ ratio even in the group of patients with NS. This aspect might be due both to a static picture of the cell subset distribution offered from this kind of study and to the heterogeneity of the disease. So, because the discovery of a numerical imbalance of T lymphocyte subsets should not constantly be considered a true sign of a pathological status of cell-mediated immunity, longitudinal studies of T cell subset distribution in the same patient and functional tests will be necessary to clarify the real role of this alteration in the pathogenesis of the disease.

Finally, no alterations in the T cell subset percentages and absolute numbers were found in IgA GN. Therefore, on the basis of our observations, the functional disorders of T cell activity in this disease reported by some authors (Sakai *et al.*, 1979; Woo *et al.*, 1981) do not seem to be due to a dysregulation of the T cell subset balance. Our data disagree with those of Chatenoud & Bach (1981) which showed an increased $OKT4^+/OKT8^+$ cell ratio in five out of the nine patients studied. However, in view of the heterogeneity of the disease (Nakamoto *et al.*, 1979), none of these findings allow us to jump to definitive conclusions about a cell-mediated immunity involvement in the pathogenesis of IgA GN.

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